From Fin Clips to Final Results

The Conservation Genetics Laboratory, Alaska Region

In the field of conservation genetics, there is a huge variety of genetic markers and analysis techniques available - and DNA can be extracted from almost any type of tissue. However, there are more similarities than differences among the various options. Here we present a generalized overview explaining how we go from fin clips to final results in the Alaska Region Conservation Genetics Laboratory.

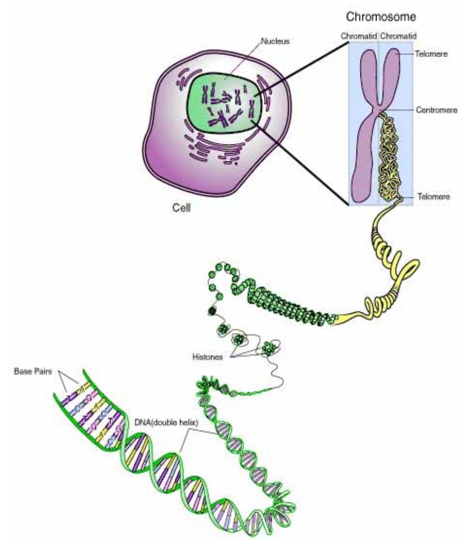
Collection

The first step in the process is acquiring tissue samples from the species being studied – this step is often the most costly and time consuming part of the process, especially in remote areas of Alaska. The samples we most often use are small clips of fin tissue stored

Sampling coho salmon on Summit Creek

in small tubes filled with high grade ethyl alcohol. These can be stored at room temperature almost indefinitely. A piece of fin tissue the size of a typical paper hole punch is more than adequate – in a pinch, we can extract DNA from samples so small you can barely see them. Once we receive samples, they are entered into our database with unique identifiers that stay with them throughout the laboratory process and data analysis.

DNA Extraction



The next step is to extract and isolate the DNA from the tissue. The primary goal of the DNA extraction process is to isolate the DNA molecules from the other cellular material. Through a series of steps using specially

Geneticists prepare samples for extractions

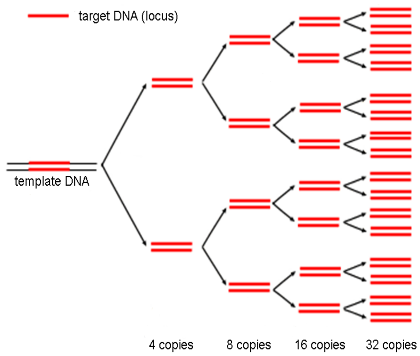
designed 96-well plates, the cell membranes and nuclei are broken down in order to release their DNA - the other cell components and extraction chemicals are then washed away. What we are left with is pure DNA – sometimes even visible with the naked eye. One lab technician can typically isolate DNA from hundreds of samples each day.

DNA Quantification

After the DNA has been isolated from the tissue, it is quantified. This process involves binding a dye to the DNA so we can visualize the amount in each well. Once we know the concentration of DNA, we add a buffer solution to dilute the sample to a standard concentration. This quality control measure allows us to be certain that each sample is at the same concentration and contains extremely clean DNA, free from compounds that may cause problems in the subsequent steps.

Polymerase Chain Reaction

The DNA is now ready for the Polymerase Chain Reaction (PCR). During the PCR process millions of copies of a target section of DNA (locus) are amplified in less than two hours. This process relies on special heat blocks that cycle between hot (95^0C) and cool (4^0C) temperatures. These high temperatures can be used because of the discovery of a heat-resistant enzyme that can copy the target DNA in a matter of hours. This discovery revolutionized the field of conservation genetics in the 1980's. Before this discovery, it took several days of cloning in bacteria to generate

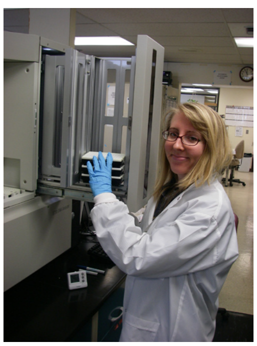


A schematic of the PCR process

sufficient copies of the target DNA. During the PCR process the target DNA is also labeled with a fluorescent tag, which allows it to be visualized in the next step.

Sequencer Analysis

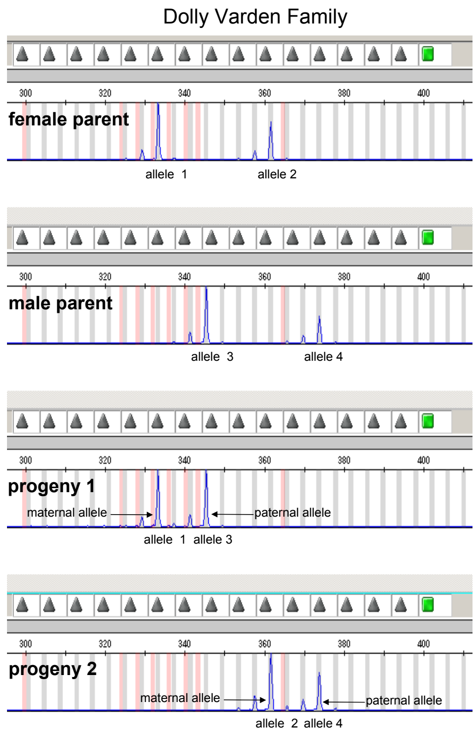
Now we are ready to move to the DNA sequencer, where we visualize the DNA locus that we just amplified and labeled.



Loading a DNA sequencer

The sequencer contains an array of tiny capillaries filled with a viscous polymer. An electrical current is passed through the capillaries in such a way that the far end of the capillaries have a net positive charge. All DNA is negatively charged. Therefore, this polarity attracts the DNA, causing it to be "pulled" through the polymer. Small fragments move faster than large ones. Therefore, the DNA fragments are separated by size. As the DNA fragments migrate toward the end of the capillaries, they pass by a stationary laser, exciting the fluorescent label, which registers an image on the internal digital camera.

For each target piece of DNA (locus), all individuals will have two copies (alleles). One allele is inherited from each parent - they may be the same size (homozygous), or they may differ (heterozygous).

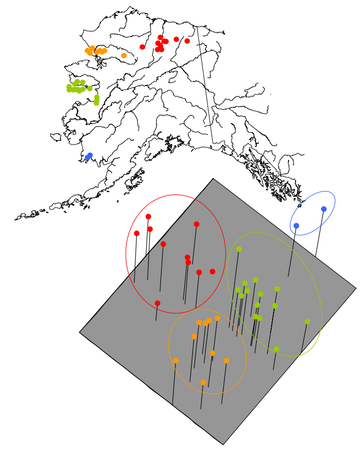


The inheritance of alleles at a single locus in a family of Dolly Varden.

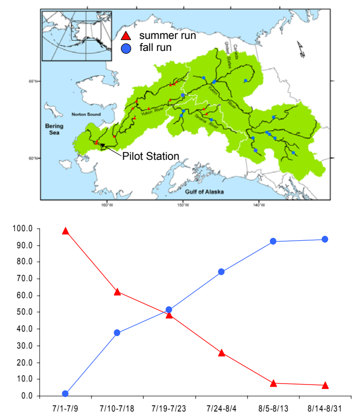
Statistical Analysis

By combining the size information for many alleles from many loci (plural of locus) we develop a genetic "fingerprint" on a variety of levels – such as individuals, populations, or species. These data are then interpreted using a variety of sophisticated statistical techniques in order to address the specific management questions at hand.

Two examples of statistical analysis typically used to address management questions



Genetic relationships among Dolly Varden populations in Northwestern Alaska



Run timing of chum salmon stocks at Pilot Station in the lower Yukon River in 2005

Reporting

Finally, our results are reported in several ways, including peer-review publications, agency reports and presentations for a variety of audiences. The process often comes full circle when we are able to personally present the results of our investigations to the residents of the Alaskan communities near where the fin clips were originally collected.

The Alaska Region Conservation Genetics Laboratory was established in 1987 as the first conservation genetics facility in the U.S. Fish & Wildlife Service (Service). The large modern facility maintains a staff of around 10 geneticists, biologists and technicians dedicated to applying conservation genetics to further the Mission of the Service.

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